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"Proteome" and "Secretome" of Trypanosomes: a standardised analytical method. (POSTER)

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During the infection in mammals, the trypanosomes are known to develop strategies to escape host immune responses that are responsible for the immunopathology. The extracellular localisation of trypanosomes implies that they use both constitutive (*i.e.* proteome) and excreted-secreted (*i.e.* secretome) factors to succeed in virulence. Proteome and secretome analysis need to be conducted in parallel to identify key trypanosome's proteins potentially involved in both virulence and pathogenicity. We developped and standardised a method to produce purified proteome and secretome of the two sub-genus Trypanozoon and Nanomonas that include all pathogenic species of trypanosomes for both human and animals (bovines, equids and camelids). The protocole is based on the production of trypanosome bloodstream forms by infection of naturally immunosuppressed rodents (Nude/SOPF®) with either *Trypanosoma brucei s.l., T. evansi* or *T.congolense*. Parasites are collected at a defined parasiteamia following the classical method of Lanham and Godfrey and incubated in a defined secretion medium that mimics the blood biochemical environment deprived of cells and macromolecules. Supernatants representing secretome are separated from parasites pellets representing proteome by centrifugation and filtration and both are conditioned for further analysis in two-dimensional gel electrophoresis and mass spectrometry for molecular characterisation or in immunocell biology experiments for functional characterisation. A preliminary comparative study was performed with referenced trypanosome strains and conducted on secretomes purified either in classical glucose phosphate buffer, classically used for bloodstream forms purification, or in defined secretion medium. One-dimensional profiles shown a high correlation at the qualitative level but the defined secretion medium revealed a higher homogeneity at the quantitative level, probably due to reduced expression of stress proteins. Two-dimensional difference gel electrophoresis (2-D DIGE) analysis of secretomes confirmed both the differences observed in 1-D gels and the high reproducibility between secretome batches of a same trypanosome strain. This standardised method thus represents a new strong tool to investigate and compare, directly at the molecular level, field isolates of trypanosome bloodstream forms to further identify new diagnostic or therapeutic molecular targets.